

CURRENT STATUS OF CLAIMS WITH CLAIM AMENDMENTS

Claims 1 to 34. **Canceled.**

35. **(Presently amended)** A method of determining protease activity of botulinum toxin serotype A or serotype E (BoNT/A/E), comprising the steps of:

(a) treating a sample, under conditions suitable for clostridial toxin protease activity, with a BoNT/A or BoNT/E substrate comprising

(i) a donor fluorophore;

(ii) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and

(iii) a BoNT/A or BoNT/E recognition sequence comprising a cleavage site,

wherein said cleavage site intervenes between said donor fluorophore and said acceptor and wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor, wherein said BoNT/A substrate has a length of amino acids selected from the group consisting of 19 amino acids, 20 amino acids, 21 amino acids, 22 amino acids, 69 amino acids and 72 amino acids;

(b) exciting said donor fluorophore; and

(c) determining resonance energy transfer of said treated substrate relative to a control substrate,

wherein a difference in resonance energy transfer of said treated substrate as compared to said control substrate is indicative of BoNT/A or BoNT/E protease activity.

36. **(Original)** The method of claim 35, wherein said botulinum toxin substrate is a BoNT/A substrate comprising a BoNT/A recognition sequence.

37. **(Withdrawn)** The method of claim 35, wherein said botulinum toxin substrate is a BoNT/E substrate comprising a BoNT/E recognition sequence.

38. **(Original)** The method of claim 35, wherein said sample is a crude cell lysate.

39. **(Previously presented)** The method of claim 35 or 36, wherein said sample is isolated clostridial toxin.

40. **(Previously presented)** The method of claim 35 or 36, wherein said sample is isolated clostridial toxin light chain.

41. **(Original)** The method of claim 35, wherein said sample is a formulated clostridial toxin product.

42. **(Previously presented)** The method of claim 35, wherein said sample is formulated BoNT/A product containing human serum albumin.

43. **(Original)** The method of claim 35, step (c) comprising detecting donor fluorescence intensity of said treated substrate,

wherein increased donor fluorescence intensity of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

44. **(Original)** The method of claim 35, step (c) comprising detecting acceptor fluorescence intensity of said treated substrate,

wherein decreased acceptor fluorescence intensity of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

45. **(Original)** The method of claim 35, step (c) comprising detecting an acceptor emission maximum and a donor fluorophore emission maximum,

wherein a shift in emission maxima from near said acceptor emission maximum to near said donor fluorophore emission maximum is indicative of clostridial toxin protease activity.

46. **(Original)** The method of claim 35, step (c) comprising detecting the ratio of fluorescence amplitudes near an acceptor emission maximum to the fluorescence amplitudes near a donor fluorophore emission maximum,

wherein a decreased ratio of said treated sample as compared to said control sample is indicative of clostridial toxin protease activity.

47. **(Original)** The method of claim 35, step (c) comprising detecting the excited state lifetime of the donor fluorophore of said treated substrate, wherein an increased donor fluorophore excited state lifetime of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

48. **(Original)** The method of claim 35, further comprising repeating step (c) at one or more later time intervals.

49. **(Previously presented)** The method of claim 35, wherein at least 90% of said BoNT/A or BoNT/E substrate is cleaved.

50. **(Previously presented)** The method of claim 35, wherein at most 25% of said BoNT/A or BoNT/E substrate is cleaved.

51. **(Previously presented)** The method of claim 50, wherein at most 15% of said BoNT/A or BoNT/E substrate is cleaved.

52. **(Previously presented)** The method of claim 51, wherein at most 5% of said BoNT/A or BoNT/E substrate is cleaved.

53. **(Original)** The method of claim 35, wherein the conditions suitable for clostridial toxin protease activity are selected such that the assay is linear.

Please add the following new claims.

54. **(New)** The method of claim 35, wherein said BoNT/A substrate has a length of 19 amino acids.

55. **(New)** The method of claim 35, wherein said BoNT/A substrate has a length of 20 amino acids.

56. **(New)** The method of claim 35, wherein said BoNT/A substrate has a length of 21 amino acids.

57. (New) The method of claim 35, wherein said BoNT/A substrate has a length of 22 amino acids.

58. (New) The method of claim 35, wherein said BoNT/A substrate has a length of 69 amino acids.

59. (New) The method of claim 35, wherein said BoNT/A substrate has a length of 72 amino acids.

60. (New) A method of determining protease activity of botulinum toxin serotype A or serotype E (BoNT/A/E), comprising the steps of:

(a) treating a sample, under conditions suitable for clostridial toxin protease activity, with a BoNT/A or BoNT/E substrate comprising

- (i) a donor fluorophore;
- (ii) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and
- (iii) a BoNT/A or BoNT/E recognition sequence comprising a cleavage site, said BoNT/A recognition sequence comprising at least six consecutive residues of human SNAP-25 (SEQ ID NO: 2), and said six consecutive residues comprising Gln₁₉₇-Arg₁₉₈ or a peptidomimetic thereof, said BoNT/E recognition sequence comprising at least six consecutive residues of human SNAP-25 (SEQ ID NO: 2), said six consecutive residues comprising Arg₁₈₀-Ile₁₈₁ or a peptidomimetic thereof,

wherein said cleavage site intervenes between said donor fluorophore and said acceptor and wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor;

- (b) exciting said donor fluorophore; and
- (c) determining resonance energy transfer of said treated substrate relative to a control substrate,

wherein a difference in resonance energy transfer of said treated substrate as compared to said control substrate is indicative of BoNT/A or BoNT/E protease activity.

61. (New) The method of claim 60, wherein said botulinum toxin substrate is a BoNT/A substrate comprising a BoNT/A recognition sequence comprising a cleavage site, said BoNT/A

recognition sequence comprising at least six consecutive residues of human SNAP-25 (SEQ ID NO: 2), said six consecutive residues comprising Gln₁₉₇-Arg₁₉₈ or a peptidomimetic thereof.

62. **(New)** The method of claim 60, wherein said botulinum toxin substrate is a BoNT/E substrate comprising a BoNT/E recognition sequence comprising a cleavage site, said BoNT/E recognition sequence comprising at least six consecutive residues of human SNAP-25 (SEQ ID NO: 2), said six consecutive residues comprising Arg₁₈₀-Ile₁₈₁ or a peptidomimetic thereof.

63. **(New)** The method of claim 60, wherein said sample is a crude cell lysate.

64. **(New)** The method of claim 60 or 61, wherein said sample is isolated clostridial toxin.

65. **(New)** The method of claim 60 or 61, wherein said sample is isolated clostridial toxin light chain.

66. **(New)** The method of claim 60, wherein said sample is a formulated clostridial toxin product.

67. **(New)** The method of claim 60, wherein said sample is formulated BoNT/A product containing human serum albumin.

68. **(New)** The method of claim 60, step (c) comprising detecting donor fluorescence intensity of said treated substrate,
wherein increased donor fluorescence intensity of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

69. **(New)** The method of claim 60, step (c) comprising detecting acceptor fluorescence intensity of said treated substrate,
wherein decreased acceptor fluorescence intensity of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

70. **(New)** The method of claim 60, step (c) comprising detecting an acceptor emission maximum and a donor fluorophore emission maximum,
wherein a shift in emission maxima from near said acceptor emission maximum to near said donor fluorophore emission maximum is indicative of clostridial toxin protease activity.

71. **(New)** The method of claim 60, step (c) comprising detecting the ratio of fluorescence amplitudes near an acceptor emission maximum to the fluorescence amplitudes near a donor fluorophore emission maximum,

wherein a decreased ratio of said treated sample as compared to said control sample is indicative of clostridial toxin protease activity.

72. **(New)** The method of claim 60, step (c) comprising detecting the excited state lifetime of the donor fluorophore of said treated substrate, wherein an increased donor fluorophore excited state lifetime of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

73. **(New)** The method of claim 60, further comprising repeating step (c) at one or more later time intervals.

74. **(New)** The method of claim 60, wherein at least 90% of said BoNT/A or BoNT/E substrate is cleaved.

75. **(New)** The method of claim 60, wherein at most 25% of said BoNT/A or BoNT/E substrate is cleaved.

76. **(New)** The method of claim 75, wherein at most 15% of said BoNT/A or BoNT/E substrate is cleaved.

77. **(New)** The method of claim 76, wherein at most 5% of said BoNT/A or BoNT/E substrate is cleaved.

78. **(New)** The method of claim 60, wherein the conditions suitable for clostridial toxin protease activity are selected such that the assay is linear.

79. **(New)** A method of determining protease activity of botulinum toxin serotype A or serotype E (BoNT/A/E), comprising the steps of:

(a) treating a sample, under conditions suitable for clostridial toxin protease activity, with a BoNT/A or BoNT/E substrate comprising

(i) a donor fluorophore;

(ii) an acceptor having an absorbance spectrum overlapping the emission

spectrum of said donor fluorophore; and

(iii) a BoNT/A or BoNT/E recognition sequence comprising a cleavage site, wherein said cleavage site intervenes between said donor fluorophore and said acceptor and wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor, wherein said donor fluorophore or said acceptor is genetically encoded;

(b) exciting said donor fluorophore; and

(c) determining resonance energy transfer of said treated substrate relative to a control substrate,

wherein a difference in resonance energy transfer of said treated substrate as compared to said control substrate is indicative of BoNT/A or BoNT/E protease activity.

80. **(New)** The method of claim 79, wherein said donor fluorophore is genetically encoded.

81. **(New)** The method of claim 79, wherein said acceptor is genetically encoded.

82. **(New)** The method of claim 79, wherein said donor fluorophore and acceptor are genetically encoded.

83. **(New)** The method of claim 79, wherein said botulinum toxin substrate is a BoNT/A substrate comprising a BoNT/A recognition sequence.

84. **(New)** The method of claim 79, wherein said botulinum toxin substrate is a BoNT/E substrate comprising a BoNT/E recognition sequence.

85. **(New)** The method of claim 79, wherein said sample is a crude cell lysate.

86. **(New)** The method of claim 79 or 83, wherein said sample is isolated clostridial toxin.

87. **(New)** The method of claim 79 or 83, wherein said sample is isolated clostridial toxin light chain.

88. **(New)** The method of claim 79, wherein said sample is a formulated clostridial toxin product.

89. **(New)** The method of claim 79, wherein said sample is formulated BoNT/A product containing human serum albumin.

90. **(New)** The method of claim 79, step (c) comprising detecting donor fluorescence intensity of said treated substrate,

wherein increased donor fluorescence intensity of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

91. **(New)** The method of claim 79, step (c) comprising detecting acceptor fluorescence intensity of said treated substrate,

wherein decreased acceptor fluorescence intensity of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

92. **(New)** The method of claim 79, step (c) comprising detecting an acceptor emission maximum and a donor fluorophore emission maximum,

wherein a shift in emission maxima from near said acceptor emission maximum to near said donor fluorophore emission maximum is indicative of clostridial toxin protease activity.

93. **(New)** The method of claim 79, step (c) comprising detecting the ratio of fluorescence amplitudes near an acceptor emission maximum to the fluorescence amplitudes near a donor fluorophore emission maximum,

wherein a decreased ratio of said treated sample as compared to said control sample is indicative of clostridial toxin protease activity.

94. **(New)** The method of claim 79, step (c) comprising detecting the excited state lifetime of the donor fluorophore of said treated substrate, wherein an increased donor fluorophore excited state lifetime of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

95. **(New)** The method of claim 79, further comprising repeating step (c) at one or more later time intervals.

96. **(New)** The method of claim 79, wherein at least 90% of said BoNT/A or BoNT/E substrate is cleaved.

97. **(New)** The method of claim 79, wherein at most 25% of said BoNT/A or BoNT/E substrate is cleaved.

98. **(New)** The method of claim 97, wherein at most 15% of said BoNT/A or BoNT/E substrate is cleaved.

99. **(New)** The method of claim 98, wherein at most 5% of said BoNT/A or BoNT/E substrate is cleaved.

100. **(New)** The method of claim 79, wherein the conditions suitable for clostridial toxin protease activity are selected such that the assay is linear.